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Erythrocyte Enzyme Activities in Cord Blood of Extremely Low-Birth-Weight Infants

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To investigate the features of erythrocyte metabolism in extremely immature infants, we assayed 21 enzyme activities and glutathione level in cord erythrocytes from 28 extremely low-birth-weight infants (ELBWI; defined as birth weight <1,000 g). The results were compared with those from normal adults and non-neonatal reticulocyte-rich controls. Statistical analysis revealed that activities of six enzymes (glucosephosphate isomerase, phosphoglycerate kinase, monophosphoglycerate mutase, enolase, glucose-6-phosphate dehydrogenase (G6PD), and glutathione reductase) were significantly higher, and those of eight other enzymes (phosphofructokinase, 6-phosphogluconate dehydrogenase (6PGD), glutathione peroxidase, adenylate kinase, adenosine deaminase, acetylcholinesterase, NADH methemoglobin reductase, and catalase) were lower in ELBWI taking their marked reticulocytosis into consideration. The 6PGD/G6PD ratio, which is consistently unchanged under various physiological and pathological conditions, was markedly reduced in ELBWI. Our results support the previous reports that neonatal erythrocytes have a unique metabolic pattern which is different from that of adult erythrocytes, and also suggest that the 6PGD/G6PD ratio might be an index for the developmental immaturity of fetal erythrocytes. This is the first report describing the pattern of erythrocyte enzyme activities in ELBWI. Am. J. Hematol 62:88–92, 1999.

Key words: extremely low birth weight infants; erythrocyte enzymes; cord blood; 6PGD/G6PD ratio

INTRODUCTION

Newborn infants are susceptible to oxidative stress and may develop Heinz body hemolytic anemia upon exposure to certain oxidants including various drugs and chemicals [1,2]. Some newborn infants develop acute Heinz body hemolytic anemia with no apparent cause [3]. We have experienced a number of such idiopathic cases, most of which are extremely low-birth-weight infants (ELBWI) [4]. Although this suggests a close relationship between idiopathic Heinz body hemolytic anemia and immature erythrocyte metabolism of ELBWI, little is known about the detailed metabolic features of these cells. To clarify the pathogenesis of idiopathic Heinz body hemolytic anemia, it is necessary to investigate the metabolic features of ELBWI erythrocytes including the patterns of enzyme activities. Although a considerable number of reports have shown that the erythrocyte enzyme activities in neonates are different from those in adults [5–14], there have been no reports concerning the pattern of enzyme activities in ELBWI erythrocytes.

Here, we describe the results of assays of 21 enzymes and reduced glutathione (GSH) level in erythrocytes from 28 ELBWI without any hematological complications. We compared these results with those from normal adults and reticulocyte-rich controls to provide basic information regarding the metabolic features of ELBWI erythrocytes.

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MATERIALS AND METHODS

Subjects

All ELBWI (defined as birth weight <1,000 g) studied were born and cared for in the Ibaraki Children's Hospital during the period from September 1994, to April 1998. Cord blood samples (6–12 ml) were collected in heparine immediately after clamping after obtaining the parents' written informed consent. The infants were admitted to the neonatal intensive care unit and their routine laboratory data including complete blood cell counts and reticulocyte counts with brilliant cresyl blue staining were monitored frequently. Of 34 ELBWI whose cord blood samples were available, we selected 28 neonates (16 males and 12 females) who were not the products of multiple pregnancies, and showed no abnormal hematological findings including Heinz body during the one month period after birth. All the infants were appropriate for their gestational age.

Controls

Heparinized blood (10 ml) was collected from 28 healthy adult volunteers after obtaining their informed consent (normal adult controls). Because ELBWI exhibit extremely high reticulocyte contents and some enzyme activities are known to be much higher in reticulocyte-rich blood, another control group (reticulocyte-rich controls) was necessary for appropriate comparison of the results. For this purpose, we selected 20 hemolytic anemia patients with reticulocytosis ranging from 13% to 62% who were studied previously in our laboratory. These subjects were older than 6 years old and had no erythrocyte enzyme defects or hematological complications other than hemolytic anemia. Their mean reticulocyte content (24.7 ± 13.4%) did not differ significantly ($p = 0.63$) from that of the ELBWI group (26.4 ± 11.1%).

Enzyme Assay

Blood samples were kept at 4°C immediately after collection to minimize the decline of red cell enzyme activities and sent to the Okinaka Memorial Institute for Medical Research, where enzyme assays were performed within 5 days. After separation from plasma and the buffy coat, erythrocytes were purified by cellulose filtration [15]. We assayed activities of 21 enzymes and glutathione level (abbreviations shown in Table II) by standard procedures [16] by using Gilford Response spectrophotometer. MetHbR and CAT were assayed in 20 ELBWI and 12 each of normal adult and reticulocyte-rich controls, and GR + FAD was assayed in 20 each of ELBWI and both controls. Other enzymes were assayed in 28 ELBWI, 28 normal adults and 20 reticulocyte-rich controls. Substrates and enzymes were purchased from Boehringer-Mannheim (Mannheim, Germany). All the other reagents were of analytical grade.

TABLE I. Profiles of ELBWI

	Mean ± SD ($n = 28$)
Gestational age (w)	25.8 ± 1.4
Birth weight (g)	798 ± 120
RBC ($10^4/mm^3$)	369 ± 60
Hb (g/dl)	14.2 ± 1.8
Ht (%)	43.7 ± 6.0
MCV (fl)	119 ± 8
MCH (pg)	38.9 ± 3.6
MCHC (%)	32.6 ± 2.1
Reticulocytes (%)	26.4 ± 11.1

Statistical Analysis

The values are expressed as means ± SD. Statistical analysis was performed by Student's *t*-test (unpaired). The data were considered significant when the *p* value was <0.05.

RESULTS

Profiles of ELBWI are summarized in Table I. The ranges of gestational age, birth weight, and the reticulocyte content were 24.4–29.1 weeks, 658–998 g, and 10–49%, respectively. Note that the reticulocyte content in ELBWI (26.4 ± 11.1%) was much higher than that in term infants (3.63 ± 1.11% [2]).

Results of assays and statistical analysis are summarized in Table 2. As a result of their marked reticulocytosis, reticulocyte-rich controls showed significantly higher activities compared with normal adults even for enzymes such as PGK, ENL, LDH, and CAT, which are not usually considered as erythrocyte age-dependent. Statistical analysis showed that ELBWI erythrocytes had significantly higher activities of GPI, PGK, MPGM, ENL, G6PD, and GR, and lower activities of PFK, 6PGD, GSH-Px, AK, ADA, AchE, CAT, and MetHbR compared with reticulocyte-rich controls. The activities of PFK, GSH-Px, AK, ADA, AchE, CAT, and MetHbR were lower in ELBWI even when compared with normal adults.

DISCUSSION

Erythrocytes in newborn infants are known to have a number of characteristic features such as a shortened survival period, increased susceptibility to oxidative stress, presence of fetal hemoglobin, increased glycolysis, and high content of ATP and other adenine nucleotides [2]. Many investigators have compared the human erythrocyte enzyme activities in cord blood [5,7–10,12–14,17] or pure fetal blood [8,17–19] with those in normal adults or reticulocyte-rich controls. It is generally accepted that fetuses and neonates show a highly consistent pattern of erythrocyte enzymes: increased activities of

TABLE II. Erythrocyte Enzyme Activities of ELBWI and Control Subjects*

	Normal adult (n = 28) mean ± SD	ELBWI (n = 28) mean ± SD	Reticulocyte-rich (n = 20) mean ± SD	ELBWI vs. reticulocyte-rich	ELBWI vs. normal adults
Hexokinase (HK)	1.05 ± 0.11	3.28 ± 0.47	3.45 ± 1.78	ns	High ($p < 0.0001$)
Glucosephosphate isomerase (GPI)	58.4 ± 5.0	83.4 ± 6.2	65.2 ± 8.5	High ($p < 0.0001$)	High ($p < 0.0001$)
Phosphofructokinase (PFK)	13.3 ± 1.7	10.1 ± 1.6	15.8 ± 2.4	Low ($p < 0.0001$)	Low ($p < 0.0001$)
Aldolase (ALD)	3.09 ± 0.53	4.73 ± 5.13	5.12 ± 0.94	ns	High ($p < 0.0001$)
Triosephosphate isomerase (TPI)	1760 ± 253	2215 ± 339	2077 ± 375	ns	High ($p < 0.0001$)
Glyceraldehyde-3-phosphate dehydrogenase (GA3PD)	191 ± 34	252 ± 39	232 ± 51	ns	High ($p < 0.0001$)
Phosphoglycerate kinase (PGK)	288 ± 31	469 ± 67	355 ± 43	High ($p < 0.0001$)	High ($p < 0.0001$)
Monophosphoglycerate mutase (MPGM)	23.5 ± 2.8	33.8 ± 6.9	29.9 ± 5.3	High ($p = 0.0412$)	High ($p < 0.0001$)
Enolase (ENL)	6.9 ± 1.0	23.9 ± 3.0	9.3 ± 1.7	High ($p < 0.0001$)	High ($p < 0.0001$)
Pyruvate kinase (PK)	16.3 ± 2.1	29.7 ± 4.0	27.6 ± 8.1	ns	High ($p < 0.0001$)
Lactate dehydrogenase (LDH)	195 ± 17	248 ± 28	264 ± 34	ns	High ($p < 0.0001$)
Glucose-6-phosphate dehydrogenase (G6PD)	7.6 ± 0.8	18.1 ± 2.2	12.8 ± 2.0	High ($p < 0.0001$)	High ($p < 0.0001$)
6-Phosphogluconate dehydrogenase (6PGD)	8.1 ± 1.0	8.2 ± 1.5	13.2 ± 1.8	Low ($p < 0.0001$)	ns
Glutathione reductase (GR)	7.1 ± 1.2	9.2 ± 1.8	7.8 ± 1.8	High ($p = 0.0090$)	High ($p < 0.0001$)
GR + flavin adenine dinucleotide (FAD)	9.9 ± 1.6[b]	10.9 ± 1.7[b]	10.4 ± 1.7	ns	ns
Glutathione peroxidase (GSH-Px)	31.8 ± 5.7	22.5 ± 4.0	41.3 ± 7.4	Low ($p < 0.0001$)	Low ($p < 0.0001$)
Adenylate kinase (AK)	252 ± 35	132 ± 18	253 ± 26	Low ($p < 0.0001$)	Low ($p < 0.0001$)
Adenosine deaminase (ADA)	1.25 ± 0.33	0.96 ± 0.30	1.60 ± 0.60	Low ($p < 0.0001$)	Low ($p = 0.0010$)
Acetylcholinesterase (AchE)	31.7 ± 4.7	16.7 ± 3.3	37.6 ± 10.1	Low ($p < 0.0001$)	Low ($p < 0.0001$)
Pyrimidine 5′-nucleotidase (P5N)	9.1 ± 1.4	17.0 ± 4.0	19.1 ± 4.5	ns	High ($p < 0.0001$)
NADH methemoglobin reductase (MetHbR)	14.9 ± 1.8[b]	11.5 ± 2.1[a]	14.1 ± 2.3[a]	Low ($p = 0.0087$)	Low ($p < 0.0001$)
Catalase (CAT)	13.6 ± 2.1[b]	11.8 ± 2.7[a]	15.6 ± 3.2[a]	Low ($p = 0.0048$)	Low ($p = 0.0459$)
Reduced glutathione (GSH) (mg/dl RBC)	67.8 ± 9.3	76.9 ± 13.8	69.4 ± 12.0	ns	High ($p = 0.0059$)

*All enzyme activities are represented as units/g Hb, except for CAT (10^4 units/g Hb) and P5N (μmol Pi liberated/hr/g Hb). ns: not significant. [a]n = 12. [b]n = 20.

ENL, PGK, GPI, and G6PD, and reduced activities of PFK, AK, and AchE. This pattern cannot be explained solely by reticulocytosis, and may be related to the unique features of fetal erythrocyte metabolism. In addition, decreased activities of antioxidant enzymes such as GSH-Px, CAT and MetHbR have been reported [8,10,20–29], although the causal relationship to increased susceptibility to oxidative stress of fetal erythrocytes is controversial [20,22,25,28,30].

There have been at least three previous reports [7,10,12] comparing erythrocyte enzyme activities in cord blood with appropriate reticulocyte-rich controls. Higher activities of PGK and ENL, and lower activities of PFK and AK in cord blood were consistently observed in all these studies, indicating that these changes might be a unique feature of fetal erythrocytes. GPI and G6PD activities in cord erythrocytes were significantly higher in ELBWI ($P < 0.0001$), whereas the activities were not consistently higher in term infants [7,12]. MPGM in ELBWI showed similar features with modest significance ($P = 0.04$). These findings are compatible with proposals that the enzyme activities may vary with gestational age [18,31].

Erythrocyte GR activity was significantly higher in ELBWI than in either control group, and similar results have previously been reported in preterm and term infants [5,6,12,13,17,21,32]. However, GR activities in ELBWI and both controls showed no significant differences when flavin adenine dinucleotide (FAD) was added into the reaction mixture (Table II). This suggested that the higher GR activity in ELBWI was not caused by the increased amount of apoenzyme, but was due to increased FAD saturation in the ELBWI enzyme.

G6PD and 6PGD are NADPH-generating enzymes in the hexose monophosphate shunt. Both enzymes are erythrocyte age-dependent, and their activities are always closely correlated under various physical and pathological conditions except for the hereditary deficiency of either enzyme (Fig. 1). It is interesting that no such correlation was observed in ELBWI erythrocytes. Increased G6PD activity in cord erythrocytes is evident even in term infants [10], while 6PGD activity in cord blood from term or slightly premature infants is similar to those in reticulocyte-rich controls or normal adults [10,13]. In contrast, our results indicated that erythrocyte 6PGD activity is significantly lower in ELBWI than in reticulocyte-rich controls, and the 6PGD/G6PD ratio is markedly reduced in those infants (Fig. 1). Because erythrocyte 6PGD activity in the fetus was reported to be significantly decreased compared with those in neonates and normal adults [17], and the calculated 6PGD/G6PD ratio in fetal erythrocytes [18] is very low, the 6PGD/G6PD ratio might represent the developmental immaturity of erythrocytes. Indeed, if defining the ratio in normal controls in each laboratory as 1.0, the corresponding ratio was calculated to be 0.38 in fetuses [18], 0.42 in ELBWI

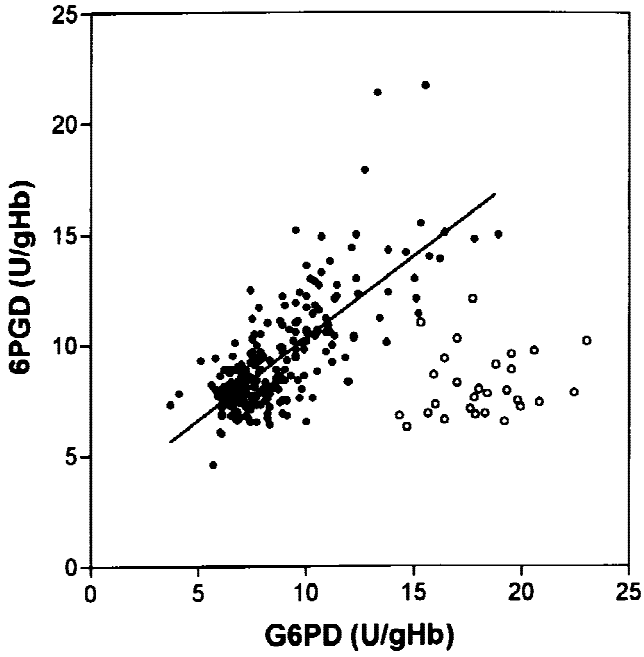

Fig. 1. Correlation between the activities of glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD) in erythrocytes from non-neonates (closed circles; $n = 233$, $r = 0.55$, $p < 0.0001$), and those in cord erythrocytes from ELBWI (open circles; $n = 28$, not significant). Non-neonates included normal adults and hemolytic anemia patients without G6PD or 6PGD deficiency.

(present report), 0.55 in slightly premature infants (27–34 weeks) [13], and 0.68 [13] and 0.85 [10] in term infants, respectively, from the published data. We also found that there was a significant positive correlation between the 6PGD/G6PD ratio and the gestational age of individual ELBWI ($r = 0.17$, $p = 0.031$; data not shown). Although it is difficult to evaluate the physiological significance of these observations at present, it is possible that the decreased 6PGD/G6PD ratio in fetal erythrocytes affects the overall hexose monophosphate shunt activity leading to increased susceptibility to oxidative stress in combination with the markedly decreased activities of GSH-Px, MetHbR, and CAT.

The normal ranges and the patterns of red cell enzyme activities in ELBWI may provide basic information for the investigation of red cell enzymopathies and other hemolytic syndromes including idiopathic Heinz body hemolytic anemia in these infants.

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